COSMETIC USE OF A BIGUANIDE DERIVATIVE AS AN ANTI-AGEING ACTIVE SUBSTANCE FOR THE SKIN

The present invention relates to the cosmetic use of a biguanide derivative as an anti-aging and restructuring agent for the skin.

In mammals in general and man in particular, the skin consists of two principal parts, namely an external layer, the epidermis and an internal layer, the dermis.

The epidermis ensures the impermeability of the skin and its resistance. It renews itself approximately every four weeks by the elimination of superficial dead cells. The epidermis is comprised principally of three types of cells, namely keratinocytes, which are in the vast majority, melanocytes and Langerhans cells. Each of these cell types contributes by its own functions to the essential role that skin plays in the organism.

The dermis provides a solid support for the epidermis. It is also its source of nourishment. It is principally constituted of fibroblasts dispersed in a complex medium, called the extracellular matrix, itself principally comprised of fibres of collagen, elastin, hyaluronic acid and proteoglycans.

Collagen represents the "cement" of the dermis. It gives the skin solidity, resistance and ensures hydration (thus its suppleness). Elastin provides elasticity and tonicity. Hyaluronic acid gives volume and participates in hydration.

As the years pass, the skin ages, first by the appearance of wrinkles, then of lines, in particular on the face, and/or of sagging skin.

Aging initially affects the epidermis, whose thickness is reduced. Basal-layer cell-division capacity decreases and regeneration time for the superficial layer of the cornea is lengthened.

Maturation of these cells is imperfect and keratinization no longer succeeds in creating a regular, homogeneous corneal

layer. Disorganization of the deep portion of the skin, slowing of cell renewal, as well as a reduction in the production of collagen and elastin are noted concomitantly.

In addition to chronobiological causes of aging, external causes exist such as the sun, tobacco, lack of sleep, and a vitamin-deficient diet.

It is also known that changes in hormone production (primarily oestrogen deficiencies) associated with menopause accentuate cutaneous aging. These changes contribute to a decrease in collagen and elastin in the dermis and to a weakening of cell renewal in the epidermis. The skin is thus less supple, less thick, and feels dry and saggy.

The structural elements of the skin (collagen, elastin, and keratin) are most responsible for our appearance. Indeed, changes in elastin and keratin levels as well as in the density of the collagen network are responsible for the loss of elasticity, wrinkles, and sagging skin. It is in part upon these which cosmetic products act.

Cosmetic products in the battle against skin aging have been known for many years. None to date, however, has been satisfactory.

Yet in a surprising way, the inventors have discovered that a biguanide derivative, advantageously metformin, has an anti-aging and restructuring effect on the epidermis and the papillary dermis, and thus on the skin.

Pharmaceutical compositions containing biguanides are already known. They are used orally to treat certain forms of diabetes and chiefly Type II noninsulin-dependent diabetes as anti-hyperglycaemic agents promoting a return to glycaemic balance.

Metformin is the biguanide derivative that is most used for this type of treatment.

This medicinal product is administered by oral route in the form of tablets containing 500 mg, 850 mg or 1 g of active ingredient.

Daily dosage is in the range between 1 and 2 g.

Phase I clinical evaluation of metformin showed the absence of toxicity of the molecule examined at hypoglycaemic doses. Tolerance to the product proved to be good and its chronic toxicity practically zero. There is no change in the behaviour or growth of animals; blood counts, uraemia and liver functions are not deteriorated.

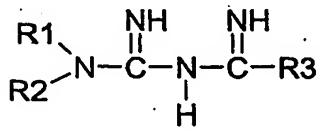
The anti-hyperglycaemic effect of metformin is attributed firstly to the increased activity of endogenous insulin and secondly to the action of metformin via insulin-independent mechanisms. The action of metformin translates as reduced intestinal absorption of glucose, increased cell absorption of blood glucose and a reduction in the liver production of glucose (elimination of neoglucogenesis) and in the quantity of insulin required to normalise glycaemia. These effects result partly from the capability of metformin to amplify the action of existing insulin through an increase in the activity of the tyrosine kinase enzyme of the insulin receptor, which triggers the 'post-receptive' signalling response.

Metformin is also known in topical compositions to promote healing and is known to have angiogenesis action (FR 2 809 310).

In addition, some biguanide derivatives are also known as having an anti-inflammatory action (US 4 163 800).

However, none of these documents describes or suggests that biguanide derivatives, in particular metformin, have a cosmetic anti-aging and restructuring action on the epidermis and the papillary dermis and that the use of a composition comprising them makes it possible to increase the production of type III collagen in the papillary dermis, to stimulate the proliferation of keratinocytes in the epidermis and/or to increase the thickness of the epidermis.

The present invention thus relates to the cosmetic use of a biguanide derivative having the following general formula I:



(I)

in which:

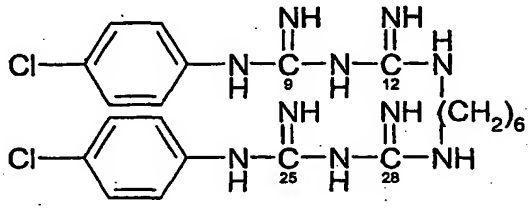
the R1 and R2 groups, independently of each other, represent a hydrogen atom, a C_1-C_7 alkyl group, a cycloalkyl group, a heterocycle, a C_2-C_7 alkenyl group, an aryl group, an aralkyl group, an aryloxylalkyl group or a heteroaryl group,

or R1 and R2 taken together represent a C_2-C_7 alkylene possibly containing one or more heteroatoms,

and the R3 group represents a primary, secondary or tertiary amine

or its cosmetically acceptable salt

with the exception of the compound of formula:



as an anti-aging and restructuring agent for the epidermis and the papillary dermis.

By the term "C_1-C_7 alkyl group" in the meaning of the present invention is meant any linear or branched C_1-C_7 group for example the methyl, ethyl, propyl, isopropyl or butyl groups and their isomers.

By the term "cycloalkyl group" in the meaning of the present invention is meant any cycloalkyl group containing 3 to 7 carbon atoms, such as the cyclohexanyl group.

By the term "heterocycle" in the meaning of the present invention is meant any cycle containing 3 to 7 atoms, one or more of these being a heteroatom such as the atom of nitrogen, oxygen or sulphur, the others being carbon atoms.

By the term "C_2-C_7 alkenyl group" in the meaning of the present invention is meant any linear or branched C_2-C_7 alkenyl group such as the vinyl or allyl groups.

By the term "aryl group" in the meaning of the present invention is meant any hydrocarbonated aromatic group such as the phenyl group for example which may contain one or more substituents such as for example a C_1-C_7 alkyl group such as defined above, a C_2-C_7 alkenyl group such as defined above or a halogen.

By the term "heteroaryl group" in the meaning of the present invention is meant any hydrocarbonated aromatic group containing one or more heteroatoms such as atoms of nitrogen, oxygen or sulphur and able to carry one or more substituents such as for example a C_1-C_7 alkyl group such as defined above, a C_2-C_7 alkenyl group such as defined above, or a halogen. Examples of heteroaryl groups are the furyl, isoxazyl, pyridyl, pyrimidyl groups.

By the term "C_2-C_7 alkylene group" in the meaning of the present invention is meant any C_2-C_7 alkylene group such as the ethylene, trimethylene, tetramethylene or pentamethylene groups for example.

By the term "cosmetically acceptable salt" in the meaning of the present invention is meant any salt prepared from any non-toxic cosmetically acceptable acid, including organic and inorganic acids. Said acids include acetic, benzenesulphonic, benzoic, citric, ethanesulphonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, lactic, maleic, malic, mandelic, methanesulphonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, tartaric and paratoluenesulphonic acid. Advantageously hydrochloric acid is used.

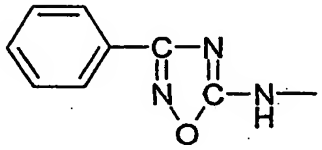
In one embodiment of the invention, the biguanide derivative according to the present invention is used to

increase the production of type III collagen in the papillary dermis, to stimulate the proliferation of keratinocytes in the epidermis and/or to increase the thickness of the epidermis.

Advantageously, this biguanide derivative has a tensor or toning effect on the skin.

It is also used to prevent, reduce and/or suppress the appearance of wrinkles on the skin.

In one particular embodiment of the invention the R3 group represents the secondary amine having the following formula:



In one advantageous embodiment of the invention the R3 group represents NH_2.

In another embodiment of the invention the R1 and R2 groups, independently of each other, represent a hydrogen atom or a C_1-C_7 alkyl group.

Advantageously, the biguanide derivative is metformin, further advantageously in the form of a hydrochloride.

In particular the derivative may be in a cosmetic composition form for local use, advantageously of oil, cream, foam, liniment, lotion, ointment, liquid, gel, milk or spray type. The forms may have a single-phase vehicle consisting of a neutral hydroxypropylcellulose gel or a gel containing sodium carboxymethylcellulose. It is also possible to prepare creams with two-phase vehicles comprising a hydrophilic phase dispersed in a lipophilic phase.

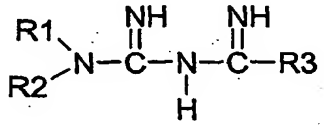
Advantageously this composition contains 0.02 to 2 % by weight of biguanide derivative having the general formula I or its cosmetically acceptable salt and a suitable excipient. These excipients may be chosen from among compounds having good compatibility with this active ingredient. They are for example hydrosoluble polymers of natural polymer type such as

polysaccharides (xanthane gum, carouba gum, peptin,..) or polypeptides, cellulose derivatives of methylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose type or further synthetic polymers, polaxamers, carbomers, PVA or PVP.

Finally, it is within the reach of all persons skilled in the art to add to this cosmetic composition various excipients of co-solvent type such as ethanol, glycerol, benzyl alcohol, humectants (glycerol) agents facilitating diffusion (transcurol, urea) or further anti-bacterial preserving agents (0.15 % methyl p-hydroxybenzoate). It may also contain surfactants, stabilizers, emulsifiers, thickeners, other active ingredients imparting a complementary or possibly synergetic effect, trace elements, essential oils, perfumes, colourings, collagen, chemical or mineral filters, hydrating agents and spa waters.

In one particular embodiment of the invention, the biguanide derivative or its cosmetically acceptable salt is combined with at least one other active ingredient.

The present invention also concerns a cosmetic treatment process of skin aging by the application of a composition comprising a biguanide derivative of the following general formula I:



(I)

in which:

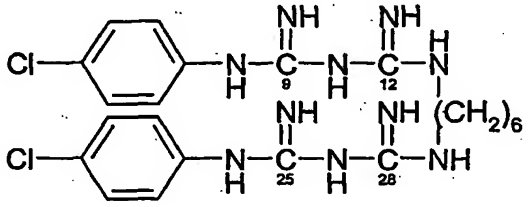
the R1 and R2 groups, independently of each other, represent a hydrogen atom, a C_1-C_7 alkyl group, a cycloalkyl group, a heterocycle, a C_2-C_7 alkenyl group, an aryl group, an aralkyl group, an aryloxylalkyl group or a heteroaryl group,

or R1 and R2 taken together represent a C_2-C_7 alkylene possibly containing one or more heteroatoms,

and the R3 group represents a primary, secondary or tertiary amine

or its cosmetically acceptable salt

with the exception of the compound of formula:



The examples given below of compositions of the invention and the examination of their activity are given by way of illustration and are not limitative.

EXAMPLES

Several pharmaceutical forms were prepared with no preserving agent. The percentages are weight percentages.

Example of formulation 1:

Metformin: 1%.

2.9 % neutral hydroxypropylcellulose gel (Klucel by Aqualon type 99 MF EP): to final 100%.

Example of formulation 2:

Metformin: 1%.

4.5 % gel containing sodium carboxymethylcellulose (Aqualon): to final 100%.

Example of formulation 3:

Metformin: 1% by weight with respect to the lipophilic phase.

33% (H/L) hydrocerin emulsion (fatty excipient by Roc® containing Vaseline, paraffin oil, triglycerides, ethers of polyoxyethylene and cerisine): to final 100%

STUDY OF THE RESTRUCTURING AND ANTI-AGING ACTIVITY OF AN OINTMENT CONTAINING METFORMIN.

The purpose of this study is to evaluate the restructuring and anti-aging activity of an ointment containing 1 % metformin (example of formulation 3) by examination of general morphology following panoptic staining and by the evaluation of the number of mitotic keratinocytes and the density of collagen III by immunolabels.

Operating mode

Preparation of explants:

On a cutaneous mammaplasty, fatty tissue removed, from a 59-year-old woman, 39 explants were prepared and maintained in survival in a culture medium. They were divided into 3 batches (ointment, excipient alone, control without treatment) of 12 explants and 1 batch of 3 explants.

Application of products:

The products to be tested (ointment and excipient alone) were applied by topical route to the explants at a rate of 4 mg per explant, for 10 days, with the untreated batches receiving no treatment.

Histology:

At time T0, 3 explants were taken and either fixed (ordinary Bouin's) or frozen. On days 3, 5, 7 and 10, 3 explants from each batch were taken and treated as previously.

The histological study was performed on:

- sections fixed with paraffin for the observation of general morphology after staining with Masson's Trichrome,
- frozen sections for the following immunolabels:
  I. Immunolabelling of mitotic cells with anti-Ki 67 monoclonal antibodies (clone 7B11) with the nuclei counterstained with propidium iodide.
  II. Immunolabelling of type III collagen with anti-collagen-III polyclonal antibody and revealed with

DAB (chromogenic revealing product for immunolabelling).

Results

1. General morphology:

Changes in cutaneous structure were sought in the various compartments, the epidermis, the dermoepidermal junction (DEJ) and the papillary dermis.

The observations made of all of the explants show that the application of ointment containing metformin induces a stimulation of epidermal structure which results in an increase in its thickness and in a more significant relief of the DEJ. This represents the image of a younger epidermal structure. Indeed, a clear epidermal acanthosis reaction (increase in the thickness of the epidermis) with a thickness up to 6 to 7 cell layers is observed beginning on treatment day 5 for the batch having received the ointment containing metformin. It should be stressed that the excipient alone has no manifest effect on the cutaneous structure of the treated explants.

2. Keratinocyte mitotic indices.

Keratinocytes in mitosis, marked by anti-Ki 67, were counted over the length of the epidermis on the sections taken.

The mitotic index, established with the values obtained, was twice as large on day 5 for the explants treated with the ointment containing metformin compared to the control explants and to those explants treated with the excipient. In addition, the mitotic index values on day 5 increased by 25 % in treated explants compared to those measured on day 3.

On day 7 and day 10, this index corresponds to the normal value for explants at this stage of survival.

These data clearly indicate that the application of the ointment containing metformin stimulates the proliferation of

keratinocytes, especially after 5 days of treatment. This observation correlates perfectly with the acanthosis visualized at the same time.

3. Type III collagen density:

Labelling the type III collagen made it possible to examine its expression in the papillary dermis and along the DEJ. The results obtained show that already on day 3, in the batch of explants having received the ointment containing metformin, collagen labelling is slightly clearer along the DEJ compared to that seen in the untreated explants and in those treated with the excipient.

From day 5 to day 10, labelling in the batch of explants having received the ointment is clearer in the papillary dermis and especially along the DEJ. These results show that application of the ointment containing metformin induces an increase in the expression of type III collagen in the papillary dermis and especially along the DEJ. This overexpression reaches its maximum on day 7 and is still quite present on day 10.

Conclusion

The application, on explants of human skin, of the ointment containing 1 % metformin for 10 days induces an increase in the thickness of the epidermis, a phenomenon more visible after 5 days of treatment but still very clearly detectable from day 7 to day 10. This acanthosis is confirmed for an increase in the mitotic index of keratinocytes which gives the ointment on day 5 values double those noted for the control and for the excipient and with an increase on the order of 25 % compared to day 3. This index returns to normal values from day 7 to day 10.

The ointment containing metformin also induces an increase in the expression of type III collagen in the papillary dermis and along the DEJ which reaches its maximum on day 7, and remains very visible on day 10.

These observations in their totality represent the stimulation of the epidermal structure, as well as the restructuring of the epidermis and the papillary dermis, by metformin. These types of changes represent the criteria for younger skin.